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## Towards cancer cell-specific phototoxic organometallic rhenium(I) complexes

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**Abstract:** Over the recent years, several Re(i) organometallic compounds have been shown to be toxic to various cancer cell lines. However, these compounds lacked sufficient selectivity towards cancer tissues to be used as novel chemotherapeutic agents. In this study, we probe the potential of two known N,N-bis(quinolinoyl) Re(i) tricarbonyl complex derivatives, namely Re(i) tricarbonyl [N,N-bis(quinolin-2-ylmethyl)amino]-4-butane-1-amine (1) and Re(i) tricarbonyl [N,N-bis(quinolin-2-ylmethyl)amino]-5-valeric acid (2), as photodynamic therapy (PDT) photosensitizers. 1 and 2 proved to be excellent singlet oxygen generators in a lipophilic environment with quantum yields of about 75%. Furthermore, we envisaged to improve the selectivity of 1 and 2 via conjugation to two types of peptides, namely a nuclear localization signal (NLS) and a derivative of the neuropeptide bombesin, to form 3 and 4, respectively. Fluorescent microscopy on cervical cancer cells (HeLa) showed that the conjugation of 1 and 2 to 3 and 4 significantly enhanced the compound's accumulation into the cell nucleus and more specifically into its nucleoli. Importantly, in view of PDT applications, the cytotoxicity of the Re complexes and their bioconjugates increased significantly upon light irradiation. In particular, 3 was found to be at least 20-fold more toxic after light irradiation. DNA photo-cleavage studies demonstrated that all compounds damaged DNA via singlet oxygen and, to a minor extent, superoxide production.

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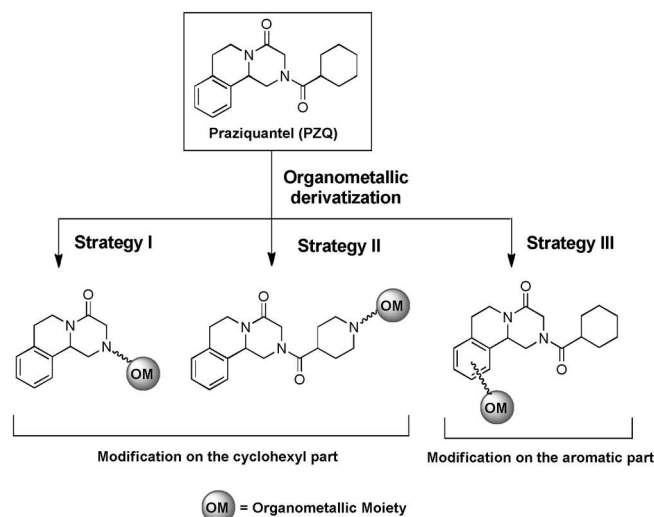
# **$[(\eta^6\text{-Praziquantel})\text{Cr}(\text{CO})_3]$ Derivatives with Remarkable In Vitro Anti-schistosomal Activity**

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Bernhard Spingler,<sup>[a]</sup> Robin B. Gasser,<sup>[d]</sup> Jennifer Keiser,<sup>\*,[b]</sup> and Gilles Gasser<sup>\*,[a]</sup>

## **$\text{Cr}(\text{CO})_3$ -Praziquantel derivatives**

**High antischistosomal activity and low toxicity**

Schistosomiasis is a major human health problem, particularly in rural, tropical regions of developing countries. Every year, 280 000 deaths are reported, mostly in sub-Saharan Africa, and more than 207 million people are infected.<sup>[1]</sup> To further emphasize the socioeconomic significance of this disease, experts consider schistosomiasis as one of the most devastating parasitic diseases after malaria in tropical countries.<sup>[2]</sup> Currently, a racemic mixture of praziquantel (PZQ, Scheme 1) is the frontline drug for treatment and control of



Scheme 1. Structures of praziquantel (PZQ) and derivatives of PZQ with organometallic moieties that were designed using three different strategies.

schistosomiasis. PZQ, which is believed to target the voltage-gated  $\text{Ca}^{2+}$  channels in the membrane of the parasite, exhibits broad-spectrum anthelmintic activity against the main species of *Schistosoma*.<sup>[3]</sup> However, reduced susceptibility of *Schistosoma mansoni* (*S. mansoni*) to PZQ has been reported recently.<sup>[4]</sup> This worrying evidence associated to the known drawbacks of PZQ, that is, low metabolic stability in

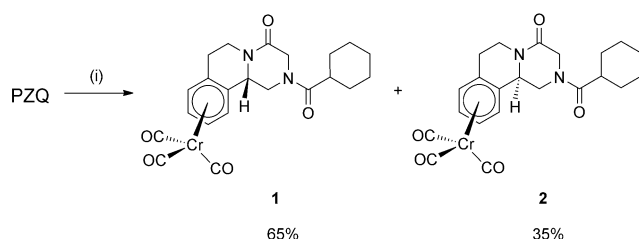
vivo<sup>[5]</sup> and lack of activity against the juvenile stage of *Schistosoma*,<sup>[3b]</sup> emphasizes the need for the rapid discovery of alternative drugs to treat schistosomiasis. With this perspective, and concurrently with the investigations of the purely organic modifications of the PZQ structure undertaken by others,<sup>[6]</sup> our groups have recently initiated a program to derivatize PZQ with organometallic moieties.<sup>[7]</sup> This strategy was shown to be very successful in the development of anti-cancer, antibacterial, and antimalarial compounds,<sup>[8]</sup> with the ferrocenyl analogue of the antimalarial drug chloroquine (CQ), namely ferroquine, being the best example. Ferroquine, which is licensed by the pharmaceutical company Sanofi, was found to be active against CQ-resistant strains of *Plasmodium falciparum* by different metal-specific modes of action.<sup>[9]</sup> Furthermore, ruthenium half-sandwich complexes were also tested for their anti-parasitic activity against *Trypanosoma cruzi* (which is responsible for Chagas disease), with promising results in vitro.<sup>[10]</sup>

In an initial study, we envisaged either replacing or modifying the cyclohexyl moiety of PZQ with different ferrocenyl derivatives (Scheme 1, Strategies I and II). Using this strategy, of eighteen ferrocenyl-PZQ derivatives made, only two showed moderate anthelmintic activity against *S. mansoni* in vitro.<sup>[7]</sup> Herein, we present an alternative strategy that employs the organometallic derivatization on the aromatic part of PZQ (Scheme 1, strategy III). For this purpose, we selected the  $\{\text{Cr}(\text{CO})_3\}$  moiety as the fragment to be attached to PZQ for following reasons. First, the preparation of  $[(\eta^6\text{-arene})\text{Cr}(\text{CO})_3]$  derivatives is usually straightforward and the resulting compounds are, in most cases, air- and water-stable. Second, we anticipated that the attachment of this organometallic moiety may improve the physicochemical properties of the parent drug (enhancement of lipophilicity, increase of metabolic stability and/or alteration of structural and electronic properties of the aromatic part of PZQ). Worthy of note, different bioorganometallic compounds containing the  $\{\text{Cr}(\text{CO})_3\}$  entity were shown earlier to be useful in field of receptorology and more recently in diverse areas of medicinal chemistry including as antimicrobial, anti-inflammatory, or antimalarial agents.<sup>[11]</sup> These findings contrast to the common believe that all chromium complexes are toxic. The toxicity of chromium complexes mainly depends on the oxidation state of the metal center, nature of the ligand, solubility, and the dosage provided. Importantly, the  $\text{Cr}^{\text{III}}$  salts that can be potentially formed by light- or oxygen-induced oxidative decomposition of  $[(\eta^6\text{-arene})\text{Cr}(\text{CO})_3]$  derivatives in solution are relatively less or non-toxic compared to the highly toxic  $\text{Cr}^{\text{VI}}$  species.<sup>[11g,12]</sup> It is also important to note that other “feared” metals, such as arsenic, antimony, gold, silver, and bismuth, have been used or are still currently being used in diverse forms in medicines.<sup>[11g,13]</sup> One of the best examples is the arsenic-containing organometallic compound Salvarsan, which was used against syphilis until the 1940s, before penicillin reached the market.<sup>[18b,13a]</sup>

In this study, the antischistosomal effects of two  $[(\eta^6\text{-PZQ})\text{Cr}(\text{CO})_3]$  ( $\text{Cr-PZQ}$ ) derivatives (**1** and **2**; Scheme 2)

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Scheme 2. Synthesis of Cr-PZQ derivatives **1** and **2**. Note that all of the compounds are racemic. Reaction conditions: i)  $[\text{Cr}(\text{CO})_6]$ ,  $\text{Bu}_2\text{O}$ , THF,  $140^\circ\text{C}$ .

were investigated. These compounds were prepared in a one-step procedure using commercially available PZQ (Scheme 2). PZQ was heated with  $[\text{Cr}(\text{CO})_6]$  at  $140^\circ\text{C}$  to yield the diastereomeric mixture of **1** and **2** in 77% combined yield (note that both **1** and **2** are racemates); **1** and **2** were found to be easily separable by silica-gel flash column chromatography. After purification, the ratio between **1** and **2** was determined to be 65 and 35%, respectively. As expected, for both diastereomers, an upfield shift in the aromatic proton signals compared with that of PZQ was observed in their  $^1\text{H}$  NMR spectrum (see the Supporting Information). ESI-MS spectra as well as the elemental analysis confirmed unambiguously the presence of the expected compounds. The designation was facilitated by the determination of the X-ray single-crystal structure of **2**. Compound **2** crystallized as a racemic mixture, and the ORTEP representation of one of the enantiomers is presented in Figure 1. The chromium tricarbonyl moiety is *trans* to the proton H18 linked to the chiral carbon C18. Furthermore, the structure of **2** does not contain any unusual structural features.<sup>[11a]</sup>

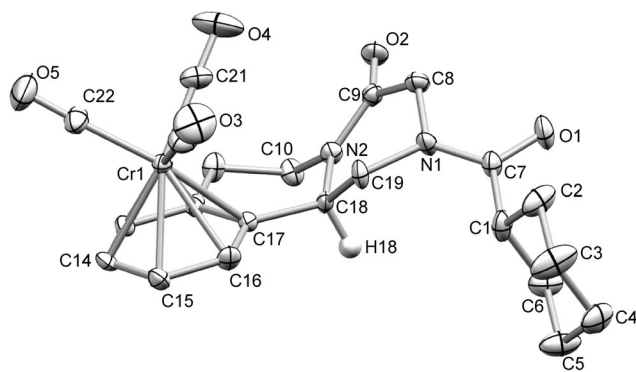


Figure 1. X-ray single-crystal structure of **2** (only one enantiomer is shown). Ellipsoids are set at 50% probability; hydrogen atoms (except  $\text{H18}$ ) are omitted for clarity.

Having the Cr-PZQ derivatives in hand, we first determined the distribution coefficients of **1** and **2** using the “shake-flask” method. The presence of chromium in **1** and **2** allows their detection in the aqueous phase by atomic absorption spectroscopy (see experimental section in the Supporting Information for further details). As expected, owing

to the presence of a  $\{\text{Cr}(\text{CO})_3\}$  moiety, the lipophilicity of both **1** ( $\log D_{7.4} = 3.49$ ) and **2** ( $\log D_{7.4} = 3.59$ ) are significantly higher than that of PZQ ( $\log D_{7.4} = 2.66$ ).<sup>[14]</sup> The membrane permeability is therefore expected to be higher for the Cr-PZQ derivatives compared to that of PZQ. The in vitro anthelmintic potential of **1** and **2** was then tested against adult *S. mansoni*, using PZQ as a control. Table 1 shows antischis-

Table 1. In vitro activity of Cr-PZQ derivatives against adult *S. mansoni* and cytotoxicity against HeLa and MRC-5 cells.

Compound	Anthelmintic activity against <i>S. mansoni</i> ( $\mu\text{M}$ )	$r^*$ <sup>[a]</sup>	IC <sub>50</sub> values ( $\mu\text{M}$ )	
			HeLa	MRC-5
<b>1</b>	0.25	0.96	$68.5 \pm 3.0$	> 100
<b>2</b>	0.27	0.97	$81.4 \pm 1.5$	> 100
<b>PZQ</b>	0.10	0.99	> 100	> 100

[a]  $r^*$  is the goodness of fit, which is required to be  $\geq 0.85$ .<sup>[15]</sup>

tosomal effects in the nanomolar range for both **1** and **2**. These antischistosomal activities were comparable to that of the parent drug PZQ ( $\text{IC}_{50} = 0.1 \mu\text{M}$ ). The toxicity of **1** and **2** on mammalian cells was then assessed on the cervical cancer (HeLa) and non-cancerous (MRC-5) cell lines (Table 1). Both compounds were moderately cytotoxic to HeLa cells but not toxic to MRC-5 cells. The high ratio (>270) calculated as the highest activity of **1** and **2** on HeLa cells ( $68.5 \mu\text{M}$ ) divided by the anthelmintic activity of **1** and **2** ( $0.25 \mu\text{M}$ ) is a good indication of the selectivity of **1** and **2** to schistosomes.

To exclude that the promising antischistosomal activities of **1** and **2** achieved in vitro were not due to the release of praziquantel, the stability of the Cr-PZQ derivatives in water was assessed. For this purpose, **1** and **2** were dissolved in a  $[\text{D}_6]\text{DMSO}/\text{D}_2\text{O}$  mixture and kept in the dark.  $^1\text{H}$  NMR spectra were then studied at different time intervals, and the presence of PZQ as a decomposition product of **1** and **2** was assessed. Very little or no PZQ was detected after two days (Supporting Information, Figures S1,S2); **1** was relatively stable and only about 8% of PZQ was released after 27 days. By comparison, **2** was shown to decompose at a higher rate (ca. 22% release in the same time). Furthermore, to further confirm the results obtained with these NMR studies, the stabilities of the Cr-PZQ derivatives in human plasma were also evaluated. Consistent with findings for the parent drug PZQ,<sup>[7]</sup> no significant change was observed either for the UV traces or the ratio between diazepam (internal standard) and **1** or **2** (Figure 2; Supporting Information, Figure S3,S4) up to 24 h, suggesting that the Cr-PZQ derivatives are relatively stable in biological medium such as serum. These results infer that chromium tricarbonyl complexes are stable and the in vitro activity was exhibited by the Cr-PZQ derivatives but not by the released PZQ.

In conclusion, we have demonstrated that two easy-to-prepare chromium tricarbonyl PZQ derivatives achieve an impressive antischistosomal effect (nanomolar range) on adult *S. mansoni* in vitro. Importantly, these compounds



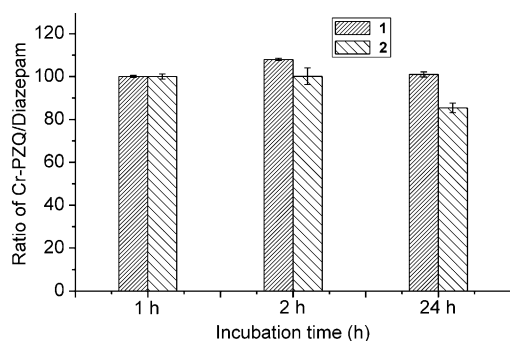


Figure 2. Ratio [%] of Cr-PZQ derivatives **1** and **2** to diazepam (internal standard) at different incubation times in human plasma.

were shown to be safe when tested using two distinct cell lines and had remarkable selectivity for the adult stage of *S. mansoni* that lives in the portal and mesenteric vein system of the human host.<sup>[16]</sup> Moreover, the stability of both compounds in human serum was confirmed by LC-MS measurements. No significant decomposition was observed when the compounds were incubated in human plasma at 37°C for 24 h. These findings therefore strongly contrast with the current belief that organometallic compounds, and more importantly, chromium tricarbonyl complexes, are unstable and/or cytotoxic. The results presented herein is another contribution to the booming field of research of medicinal organometallic chemistry<sup>[17]</sup> and pave the way for a systematic investigation of the structure-activity relationship of organometallic derivatives of PZQ as well as in vivo testing of such compounds.

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- [1] P. Steinmann, J. Keiser, R. Bos, M. Tanner, J. Utzinger, *Lancet Infect. Dis.* **2006**, *6*, 411–425
- [2] C. H. King, *Acta Trop.* **2010**, *113*, 95–104.
- [3] a) R. N. Beech, A. Silvestre, *Anti-Infect. Agents Med. Chem.* **2010**, *9*, 105–112; b) M. J. Doenhoff, D. Cioli, J. Utzinger, *Curr. Opin. Infect. Dis.* **2008**, *21*, 659–667.
- [4] a) M. Ismail, S. Botros, A. Metwally, S. William, A. Farghally, L. F. Tao, T. A. Day, J. L. Bennett, *Am. J. Trop. Med. Hyg.* **1999**, *60*, 932–935; b) S. D. Melman, M. L. Steinauer, C. Cunningham, L. S. Kubatko, I. N. Mwangi, N. B. Wynn, M. W. Mutuku, D. M. S. Karanja,

- D. G. Colley, C. L. Black, W. E. Secor, G. M. Mkoji, E. S. Loker, *PLoS Negl. Trop. Dis.* **2009**, *3*, e504.
- [5] a) J. Huang, S. P. Bathena, Y. Alnouti, *Drug Metab. Pharmacokinet.* **2010**, *25*, 487–499; b) D. Cioli, L. Pica-Mattoccia, S. Archer, *Pharmacol. Ther.* **1995**, *68*, 35–85.
- [6] a) F. Ronketti, A. V. Ramana, X. Chao-Ming, L. Pica-Mattoccia, D. Ciolic, M. H. Todd, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4154–4157; b) S. A.-L. Laurent, J. Boissier, F. Coslédan, H. Gornitzka, A. Robert, B. Meunier, *Eur. J. Inorg. Chem.* **2008**, 895–913; c) H. Liu, S. William, E. Herdtweck, S. Botros, A. Dömling, *Chem. Biol. Drug Des.* **2012**, *29*, 470–477; d) Y. Dong, J. Chollet, M. Vargas, N. R. Mansour, Q. Bickle, Y. Alnouti, J. Huang, J. Keiser, J. L. Venerstrom, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2481–2484.
- [7] M. Patra, K. Ingram, V. Pierroz, S. Ferrari, B. Spingler, J. Keiser, G. Gasser, *J. Med. Chem.* **2012**, *55*, 8790–8798.
- [8] a) G. Jaouen, S. Top, A. Vessieres, G. Leclercq, M. J. McGlinchey, *Curr. Med. Chem.* **2004**, *11*, 2505–2517; b) M. Patra, G. Gasser, N. Metzler-Nolte, *Dalton Trans.* **2012**, *41*, 6350–6358; c) P. C. Bruijninx, P. J. Sadler, *Curr. Opin. Chem. Biol.* **2008**, *12*, 197–206; and references therein; d) C. Biot, D. Dive in *Medicinal Organometallic Chemistry*, Vol. 32 (Eds.: G. Jaouen, N. Metzler-Nolte), Springer, Heidelberg, **2010**, pp. 155–193.
- [9] C. Biot, W. Castro, C. Y. Botte, M. Navarro, *Dalton Trans.* **2012**, *41*, 6335–6349.
- [10] a) A. Martínez, T. Carreon, E. Iniguez, A. Anzellotti, A. Sánchez, M. Tyan, A. Sattler, L. Herrera, R. A. Maldonado, R. A. Sánchez-Delgado, *J. Med. Chem.* **2012**, *55*, 3867–3877; b) T. Küster, N. Lense, F. Barna, A. Hemphill, M. K. Kindermann, J. W. Heinicke, C. A. Vock, *J. Med. Chem.* **2012**, *55*, 4178–4188.
- [11] a) M. Patra, K. Merz, N. Metzler-Nolte, *Dalton Trans.* **2012**, *41*, 112–117; b) H. Bielig, J. Velder, A. Saiai, M. Menning, S. Meemboor, W. Kalka-Moll, M. Krönke, H.-G. Schmalz, T. A. Kufe, *ChemMedChem* **2010**, *5*, 2065–2071; c) L. Glans, D. Taylor, C. d. Kock, P. J. Smith, M. Haukka, J. R. Moss, E. Nordlander, *J. Inorg. Biochem.* **2011**, *105*, 985–990; d) G. Jaouen, A. Vessieres, S. Top, A. A. Ismail, I. S. Butler, *J. Am. Chem. Soc.* **1985**, *107*, 4778–4780; e) A. Vessieres, S. Top, A. A. Ismail, I. S. Butler, M. Louer, G. Jaouen, *Biochemistry* **1988**, *27*, 6659–6666; f) S. Top, A. Vessieres, G. Jaouen, *J. Labelled Compd. Radiopharm.* **1987**, *24*, 1257–1263; g) M. Patra, G. Gasser, A. Pinto, K. Merz, I. Ott, J. E. Bandow, N. Metzler-Nolte, *ChemMedChem* **2009**, *4*, 1930–1938.
- [12] L. Assem, H. Zhu, *Chromium Toxicological Overview*; Health Protection Agency, **2007**, [http://www.hpa.org.uk/webc/HPAwebFile/HPAweb\\_C/1194947362170](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1194947362170).
- [13] a) S. Gibaud, G. Jaouen in *Medicinal Organometallic Chemistry* (Eds.: G. Jaouen, N. Metzler-Nolte), Springer, Berlin, **2010**, pp. 1–20, and references therein; b) *Metallotherapeutic Drugs and Metal-Based Diagnostic Agents, The Use of Metals in Medicine* (Eds.: M. Gielen, E. R. T. Tiekink), Wiley, Chichester, **2005**; c) S. J. Berners-Price in *Bioinorganic Medicinal Chemistry* (Ed.: E. Alessio), Wiley-VCH, Weinheim, **2011**, pp. 197–222.
- [14] <https://www.ebi.ac.uk/chembl/index.php/compound/inspect/CHEMBL976>.
- [15] T.-C. Chou, *Cancer Res.* **2010**, *70*, 440–446.
- [16] B. Gryseels, K. Polman, J. Clerinx, L. Kestens, *Lancet* **2006**, *368*, 1106–1118.
- [17] a) See Ref. [8c], and references therein; b) C. Hartinger, P. J. Dyson, *Chem. Soc. Rev.* **2009**, *38*, 391–401; c) C. G. Hartinger, N. Metzler-Nolte, P. J. Dyson, *Organometallics* **2012**, *31*, 5677–5685; d) G. Gasser, N. Metzler-Nolte, *Curr. Opin. Chem. Biol.* **2012**, *16*, 84–91; e) U. Schatzschneider, N. Metzler-Nolte, *Angew. Chem.* **2006**, *118*, 1534–1537; *Angew. Chem. Int. Ed.* **2006**, *45*, 1504–1507, and references therein; f) G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3–25; g) *Topics in Organometallic Chemistry*, Vol. 32, 1st ed. (Eds.: G. Jaouen, N. Metzler-Nolte), Springer, Heidelberg, **2010**, and references therein.

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